Docket No. 57094 (71526)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	M. Sakanaka et al.
Serial No.	:	10/070,209
Filed	:	February 28, 2002
For	:	Brain Cell- or Nerve Cell- Protecting Agents Comprising Medicinal Ginseng
Examiner	:	Patricia Leith
Art Unit	:	1654

Mail Stop: Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

DECLARATION UNDER 37 C.F.R. 1.132

I, Masahiro Sakanaka, a citizen of Japan residing at 1191-13 Oaza Shitsukawa, Shigenobu-cho, Onsen-gun, Ehime 791-0204, Japan, hereby declare as follows:

1. I am a co-inventor of the subject matter described and claimed in the patent application U.S.S.N. 10/070,209, filed on February 28, 2002 and otherwise identified above.

2. I have read and understood the Final Office Action dated August 26, 2004, the references cited in the Final Office Action and the later issued Advisory Action dated

December 2, 2004 in the above case.

3. The following experiments were conducted by me or under my supervision, to compare the therapeutic effect of ginsenoside Rb1 (which is one of the ginsenoside compositions) administered in low-dosage according to the present invention with that of ginsenoside Rb1 administered in high-dosage.

(I) Experiment:

Male SH-SP rats (spontaneous hypertensive stroke-prone rats, weighing about 300 g), at the age of 12-15 weeks, were used. Animals were bred in a room furnished with 12 hours light and dark cycles and water and feeds were supplied ad libitum. The cortical branch of the left middle cerebral artery (MCA) of each animal was coagulated and cut under inhalation anesthesia. 2 Hours after permanent MCA occlusion, ginsenoside Rb1 dissolved in physiological saline was infused once intravenously (6 μg, 60 μg, 3 mg or 12 mg), and subsequently intravenously infused in a continuous manner for 24 hours by using an Alza osmotic minipump. The amount of ginsenoside Rb1 administered continuously per 24 hours was 6 μg, 60 μg, 3 mg or 12 mg (n=6-8 for each group), and that is converted to 20 μg/kg/day, 200 μg/kg/day, 10 mg/kg/day and 40 mg/kg/day respectively. Further, ginsenoside Rb1 was all dissolved in physiological saline and infused into the left femoral vein. Control animals with permanent MCA occlusion (ischemic control animals) were intravenously infused with the same amount of physiological saline (vehicle) (n=8).

At 26 hours after MCA permanent occlusion, a lethal dose of pentobarbital was injected intraperitoneally into rats. Immediately after animals were put away, the brains were dissected out and seven frontal sections 2 mm thick were prepared. The sections were immersed in 1% 2,3,5-triphenyl-tetrazolium chloride (TTC) solution for 30 minutes at 37 °C and fixed with 10% formalin for more than 12 hours. The border between infarcted and non-infarcted tissue was outlined with an image analysis system, and the area of

infarction was measured by subtracting the area of the non-lesioned ipsilateral hemisphere from that of the contralateral hemisphere (Swanson RA, Morton MT, Tsao WG, Savalos RA, Davidson C, Sharp FR (1990) A semiautomated method for measuring brain infarct volume. Journal of Cerebral Blood Flow & Metabolism 10:290-293). The volume of infarction was calculated by integration of the lesion areas at seven equidistant levels of forebrain.

(II) Experimental Result:

Results are shown in Figs.1, 2 and 3 of reference data. Fig. 1 shows a case administered with physiological saline, 20 μg/kg/day (6 μg/day) of ginsenoside Rb1 and 200 μg/kg/day (60 μg/day) of ginsenoside Rb1, respectively. Fig. 2 shows a case administered with physiological saline, 10 mg/kg/day (3 mg/day) of ginsenoside Rb1 and 40 mg/kg/day (12 mg/day) of ginsenoside Rb1, respectively. Fig. 3 is a chart which shows infarct volume of physiological saline group and ginsenoside Rb1 group (four groups).

As shown in the left column of Fig. 1, in the rats administered with physiological saline alone 2 hours after MCA permanent occlusion, cerebral infarct lesions, which were not stained with TTC, were observed with white color in the cerebral cortex on the left side. On the other hand, as shown in the middle and the right columns of Fig. 1, in the rats administered intravenously with ginsenoside Rb1 in low-dosage, i.e. 20 μg/kg/day (6 μg/day) or 200 μg/kg/day (60 μg/day), 2 hours after MCA permanent occlusion, cerebral infarct lesions were markedly reduced, compared to that of rats administered with physiological saline. Therefore, it was ascertained that there is at least 2 hours of therapeutic time window in low-dosage ginsenoside Rb1.

As shown in the left column of Fig. 2, in the rats administered with physiological saline alone 2 hours after MCA permanent occlusion, cerebral infarct lesions, which were not stained with TTC, were observed with white color in the cerebral cortex on the left side as is the case with Fig.1. On the other hand, as shown in the middle and the right columns

of Fig. 2, in the rats administered intravenously with ginsenoside Rb1 in high-dosage, i.e. 10 mg/kg/day (3 mg/day) or 40 mg/kg/day (12 mg/day), 2 hours after MCA permanent occlusion, no improvements in the reduction of cerebral infarct lesions were identified compared to that of rats administered with physiological saline. In Fig. 2, the most rostral section containing no infarct lesion was deleted from each column.

From this result, contrary to the expectation of one of ordinary skill in the art, therapeutic effect of high-dosage ginsenoside Rb1 is obviously inferior to that of low-dosage ginsenoside Rb1. This is highly surprising.

The results described in Fig.1 and Fig.2 are illustrated in Fig.3. As shown in Fig.3, low-dosage ginsenoside Rb1 administered intravenously 2 hours after permanent MCA occlusion were able to reduce significantly cerebral infarct lesions compared to that of physiological saline. On the contrary, it was proved from Fig.3 that high-dosage ginsenoside Rb1 administered intravenously 2 hours after permanent MCA occlusion did not show therapeutic effect on cerebral infarction at all.

Generally, it is recognized in the clinical field that it takes at least 1-2 hours from the onset of MCA permanent occlusion for the patients to reach hospital or medical institute. Therefore, the length of the therapeutic time window is a major criterion for the remedy of cerebral infarction. According to the present experiment, it is proved that the low-dosage ginsenoside Rb1 is obviously superior to high-dosage ginsenoside Rb1 in terms of clinically applicable therapeutic time window. It was solely discovered by us (the inventors of the present invention) that the advantageous effect of low-dosage ginsenoside Rb1 is surprisingly superior to that of high-dosage ginsenoside Rb1. This discovery was not obvious to one of ordinary skill in the art, even in view of the cited reference. Therefore, we (the inventors of the present invention) declare that the present invention concerning the therapeutic effect of low-dosage ginsenoside compositions (especially ginsenoside Rb1) is inventive and non-obvious.

4. I, the undersigned Masahiro Sakanaka, further declare that all statements made herein of my own knowledge are true and that all statements made upon information and belief are believed to be true, and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both under Section 101 of Title 18 of the United States Code and that such willful false statement may jeopardize the validity of the above identified application or any patent issuing thereon.

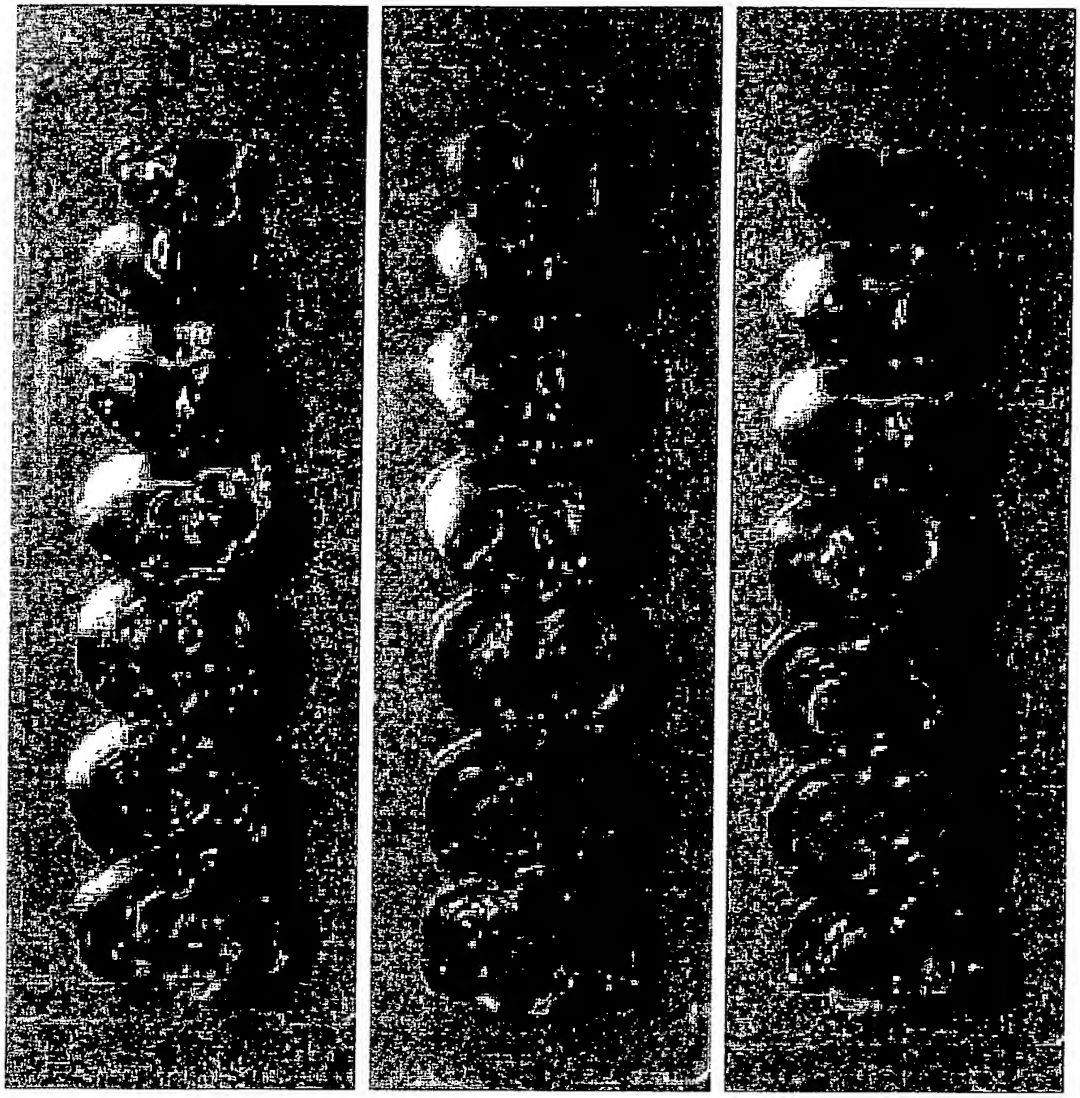
By: M. Sakanaka

Masahiro Sakanaka

Date: February 18, 2005

参考資料図１

Intravenous Rb-1 infusion starting 2 hour after permanent MCA occlusion



Saline　　20μg/kg/day (6μg/day)　　200μg/kg/day (60μg/day)

Rb-1

参考資料図２

Intravenous Rb−1 infusion starting 2 hour after permanent MCA occlusion

Saline

10mg/kg/day
(3mg/day)

40mg/kg/day
(12mg/day)

Rb-1

参考資料図３